

Allozyme diversity of Russian wildrye accessions

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With 1 figure and 4 tables

Received July 29, 2007/Accepted January 17, 2008

Communicated by O. A. Rognli

Abstract

Analysis of genetic diversity among plant accessions plays an important role in developing strategies for plant breeding and the conservation of genetic resources. Therefore, we sought to estimate the extent of genetic diversity present among and within 29 Russian wildrye accessions, which were collected from widespread geographic sites. We assessed the genetic diversity of eight enzymes found in 29 Russian wildrye accessions using vertical polyacrylamide gel electrophoresis. The genetic diversity of each accession was estimated from standard genetic parameters that included (1) the number of polymorphic loci; (2) the average number of alleles per locus; (3) the observed and expected heterozygosity. The eight enzymes were encoded by 13 putative loci and 46 alleles. Allozyme analyses revealed a large number of polymorphic loci among the 29 accessions and some of the accessions carry rare alleles and trace their origin to sites in Xinjiang, China, where Russian wildrye is indigenous. The accessions with rare alleles require additional evaluation to determine whether they possess unique phenotypic traits which would be useful in applied plant breeding.

Key words: Russian wildrye — allozyme diversity — genetic diversity — polymorphism — plant breeding — plant conservation

Russian wildrye [*Psathyrostachys juncea* (Fischer) Nevski] is an indigenous grass of the Eurasian interior. *Psathyrostachys* is a small genus of the tribe Triticeae and consists of no more than 10 species described by Nevski (Dewey 1984). In this genus, Russian wildrye is the only species, which has been recognized as a forage grass. The plant is a cross-pollinated, highly self-sterile, long-lived, perennial bunchgrass (Jensen et al. 1990) and is tolerant to alkalinity and drought. Furthermore, Russian wildrye is often used for fall and early-winter grazing when the nutritive quality value of other forage grasses is relatively low (Bai et al. 1999, Huo et al. 2001).

Analysis of genetic diversity among plant accessions plays an important role in developing strategies for plant breeding and the conservation of genetic resources (Simioniue et al. 2002; Dean et al. 1999). Quantifying the extent of genetic diversity within a plant species is especially useful for characterizing individual accessions and cultivars and in detecting duplications of genetic material in germplasm collections. Quantifying genetic diversity can also aid the selection of parents for breeding hybrids to maximize genetic gain (Davila et al. 1998; Ribaut and Hoisington 1998). A diverse genetic background among parental lines provides an ample supply of allelic variation that can be used to create new favourable gene

combinations. In the past, indirect estimates of genetic diversity, which were based on morphological and agronomic information, have been widely used in many species, including *Psathyrostachys juncea* (Berdahl et al. 1999). However, morphological variation sometimes does not reliably reflect the true extent of genetic variability because of genotype–environment interactions and the largely unknown genetic control of polygenically inherited morphological and agronomic traits (Smith and Smith 1992).

In recent years, a number of genetic techniques, such as the use of allozyme markers, have become available for studying genetic diversity at the molecular level. Allozyme data have been widely used to detect and measure genetic diversity within and among populations (Huh 2001, Fu and Dane 2003) because the technique of allozyme separation and visualization is cheap and relatively rapid (Hamrick and Godt 1997).

Current Russian wildrye cultivars have a narrow genetic base (Berdahl et al. 1999) and there is limited information on the genetic diversity of Russian wildrye at the molecular level. William and Mujeeb-Kazi (1992) reported that isozyme markers could be used to tracking Russian wildrye chromosomes in wheat. Wei et al. (1996) analysed 10 allozymes to measure the genetic diversity within and among 11 Russian wildrye accessions from different geographic areas.

Genetic gain in a selected species is dependent on the extent of genetic diversity in the founder members used in a breeding programme. This study had two objectives. Firstly, we sought to estimate the extent of allozyme diversity present among and within 29 Russian wildrye accessions which were collected from widespread geographic sites. Secondly, we aimed to measure the genetic distance among these accessions using an unweighted pair group method with arithmetic mean (UPGMA) cluster analysis.

Materials and Methods

Plant material: We analysed the genetic diversity of 29 germplasm lines of Russian wildrye which comprised 7 synthetic populations, 3 cultivars and 19 wild populations (Table 1). The synthetic populations were generously supplied by USDA-ARS (Mandan, ND, USA). The cultivars included Mankota (Berdahl et al. 1992) (generously supplied by USDA-ARS, Mandan, ND, USA), Ziniquan (Shali 1997) and Shandan (Sun et al. 2000). Thirteen of the 19 wild populations were generously provided by the Grassland Institute of the Chinese Academy of Agricultural Sciences (CAAS) and six populations were collected for this project in August 2004. Randomly chosen seedlings

Table 1: The accessions of Russian wildrye used in the present study

No.	Accession	Identity	Origin or source
1	Synthetic 1802		Provided by USDA-ARS
2	Synthetic 1805		Provided by USDA-ARS
3	Synthetic 1982		Provided by USDA-ARS
4	Mankota	Cultivar	Provided by USDA-ARS
5	Synthetic 1881		Provided by USDA-ARS
6	Synthetic 1808		Provided by USDA-ARS
7	Synthetic 1981		Provided by USDA-ARS
8	Synthetic 1831		Provided by USDA-ARS
9	02472	Wild accession	Origin unknown, provided by CAAS
10	00819	Wild accession	Origin unknown, provided by CAAS
11	00818	Wild accession	Origin unknown, provided by CAAS
12	00697	Wild accession	origin unknown, provided by CAAS
13	02474	Wild accession	Canada, provided by CAAS
14	00289	Wild accession	Canada, provided by CAAS
15	00293	Wild accession	Canada, provided by CAAS
16	02471	Wild accession	China, Xinjiang, provided by CAAS
17	00291	Wild accession	China, Xinjiang, Provided by CAAS
18	01043	Wild accession	China, Xinjiang, provided by CAAS
19	01022	Wild accession	China, Xinjiang, Provided by CAAS
20	X94-004	Wild accession	China, Xinjiang, provided by CAAS
21	X97-149	Wild accession	China, Xinjiang, provided by CAAS
22	Shandan	Cultivar	China
23	X1	Wild accession	China, Xinjiang
24	X2	Wild accession	China, Xinjiang
25	X3	Wild accession	China, Xinjiang
26	X4	Wild accession	China, Xinjiang
27	X5	Wild accession	China, Xinjiang
28	Ziniquan	Cultivar	China, Xinjiang
29	X7	Wild accession	China, Xinjiang

from each accession were transplanted in a field nursery located at the Guyan Farm, Heibei Province, P.R. China (41°56' N, 115°47' W) on May 10, 2005. Ten individuals in each population were analysed for allozyme pattern.

Allozyme electrophoresis: Young leaves of each plant were collected on July 10, 2005 and stored at 4°C. Within 4 days of collection, approximately 1.0 g of leaf tissue was ground in 4 ml of Tris-HCl extraction buffer [0.1 M Tris-HCl, pH 7.0, 0.001 M EDTA, 0.01 M KCl, 0.01 M MgCl₂, 4% polyvinylpyrrolidone (w/v), 0.1% 2-mercaptoethanol(v/v); (Soltis et al. 1983)] on an ice plate. The extracts were then transferred to 5 ml centrifuge tubes and centrifuged at 10 000 *g* for 15 min at 4°C. Following centrifugation, the supernatant was mixed with an identical volume of sucrose solution (400 g/l) and stored at -80°C, pending analysis. Allozyme diversity was determined using vertical polyacrylamide gel electrophoresis according to the method described by Yang and Wu (1999). The activities of eight enzymes were determined in two buffer systems (Table 2), according to the methods described by Soltis et al. (1983) and Wendel and Weeden (1989).

Data analysis: In cases where an enzyme had more than one zone of activity, the isozyme closest to the anode was arbitrarily designated as '1' and the other isozymes were sequentially assigned higher numbers. Similarly, alleles were identified sequentially with the allozyme closest to the anode designated as 'a' and the progressively slower forms designated 'b', 'c' and so on. The genetic diversity of each accession was

Table 2: Enzymes, loci and the numbers of alleles detected in the 29 accessions of Russian wildrye

Enzyme	Abbreviation	E.C. no.	Locus	No. of allele detected
Aspartate aminotransferase	AAT	2.6.1.1	<i>Aat-1</i>	3
			<i>Aat-2</i>	2
Glucose-6-phosphate dehydrogenase	G6PD	1.1.1.49	<i>G6pd-1</i>	4
			<i>G6pd-2</i>	2
Malate dehydrogenase	MDH	1.1.1.37	<i>Mdh</i>	2
Malic enzyme	ME	1.1.1.40	<i>Me</i>	5
Phosphogluconate dehydrogenase	PGD	1.1.1.44	<i>Pgd-1</i>	5
			<i>Pgd-2</i>	4
Shikimate dehydrogenase	SKD	1.1.1.25	<i>Skd</i>	5
Esterase	EST	3.1.1.-	<i>Est-1</i>	4
			<i>Est-2</i>	5
Peroxidase	POD	1.11.1.7	<i>Pod-1</i>	2
			<i>Pod-2</i>	3

estimated using Popgene version 1.32 (Yeh et al. 1999) from various standard genetic parameters. The genetic parameters included (1) the percentage of polymorphic loci (*P*); (2) the effective number of alleles per locus (*A_e*); (3) the average number of alleles per locus (*A*); (4) the observed heterozygosity (*H_o*) and (5) the expected heterozygosity (*H_e*). The value of *P*, *H_o* and *H_e* were calculated at the species (subscript 's') and population level (subscript 'p').

To describe diversity structure, the total allelic diversity (*H_T*), the mean allelic diversity within the accessions (*H_S*) and the mean genetic diversity among accessions (*D_{ST}*) were calculated using the method described by Nei (1973). The ratio *D_{ST}* : *H_T*, was used to calculate the coefficient of gene variations found among populations (*G_{ST}*) (Nei 1977). Estimates of the number of migrants per generation (*N_m*) were based on *G_{ST}*: *N_m* = (1 - *G_{ST}*)/4*G_{ST}*. The diversity structure within and among populations was also evaluated using Wright's *F*-statistics (Wright 1965). *F_{IS}* and *F_{IT}* measure heterozygote excess (<0) and deficit (>0) relative to the Hardy-Weinberg expectation in local populations and the total population, respectively. *F_{ST}* represents the proportionate genetic differentiation between the populations. The genetic identity (*I*) and genetic distances (*D*) were computed according to a previously described method (Nei 1978). Cluster analysis, based on genetic distances, was carried out using a computer program for numerical taxonomy and multivariate analysis, NTSYSpc 2.1 (Rohlf 1992). A phenogram was constructed using UPGMA and making use of the sequential, agglomerative, hierarchical and nested clustering (SHAN) routine in the NTSYS program (Sneath and Sokal 1973).

Results

Allele frequencies

The eight enzymes were encoded by 13 putative loci and 46 alleles (Table 2). Five enzymes, AAT, G6PD, PGD, EST and POD had two loci; all other enzymes had one locus each. Except for MDH, the 13 loci were polymorphic. The number of alleles at each locus ranged from two to five. Loci *Me*, *Pgd-1*, *Skd* and *Est-2* had five alleles, whereas the loci, *Aat-2*, *G6pd-2*, *Mdh* and *Pod-1*, contained two alleles. We identified three rare alleles, *aat-1c*, *mdhb* and *est-1d*, none of which had been previously identified in Russian wildrye (Wei et al. 1996). The *aat-1c* allele was detected in accessions 19 and 26, both of which were wild accessions from Xinjiang, China. The alleles, *mdhb* and *est-1d*, were present only in accession 29, which was also from Xinjiang, China.

The 29 accessions displayed different levels of allele polymorphism. Among the 29 accessions, no accession harbored all 46 alleles. Accessions 1, 11 and 22 each contained 41 alleles or 89.1% of the total number of alleles. Although accessions 19,

26 and 29 harbored rare alleles, these accessions did not have the highest number of alleles. Accession 23, another wild accession from Xinjiang, China, contained 33 alleles, which was the lowest number of alleles found in the 29 accessions.

Estimates of genetic diversity

The percentage of polymorphic loci (P), the average number of alleles per locus (A), the effective number of alleles per locus (A_e) and the expected heterozygosity (H_e) were used to estimate genetic diversity. The genetic diversity of the 29 accessions of Russian wildrye is summarized in Table 3. All accessions had high polymorphism. The mean percentage of polymorphic loci (P) was 81.2% and 100% at the population and species levels, respectively. The percentage of polymorphic loci (P) ranged from 76.9% for accessions 8 and 9 to 92.3% for several accessions. The effective number of alleles per locus (A_e) ranged from 2.03 to 2.68 with a mean of 2.39. The expected heterozygosity (H_e) ranged from a minimum value of 0.451, which was detected in accession 6, a six-parent synthetic accession, to a maximum value of 0.556 in accession 22 which had been collected from Xinjiang, China. The mean expected heterozygosity estimated at the population and species level was 0.521 and 0.557, respectively. The mean observed heterozygosity (H_o) estimated at the population and species level was 0.640.

Diversity structure

The distribution of genetic diversity was estimated using Nei's statistics (Nei 1973, 1978). Distributions of the total allelic

Table 3: Genetic diversity statistics for 29 accessions of Russian wildrye

Accession	P	A	A_e	H_o	H_e
1	92.3	3.15	2.48	0.677	0.541
2	92.3	2.85	2.24	0.623	0.519
3	84.6	3.00	2.41	0.600	0.519
4	92.3	3.15	2.42	0.585	0.527
5	84.6	2.92	2.41	0.554	0.526
6	92.3	2.62	2.03	0.585	0.451
7	84.6	2.85	2.26	0.539	0.487
8	76.9	3.00	2.21	0.562	0.470
9	76.9	3.00	2.29	0.592	0.490
10	84.6	3.00	2.31	0.585	0.508
11	84.6	3.15	2.58	0.662	0.535
12	84.6	3.08	2.48	0.654	0.533
13	84.6	2.85	2.43	0.600	0.512
14	84.6	3.00	2.55	0.685	0.537
15	84.6	3.00	2.07	0.631	0.558
16	92.3	2.85	2.42	0.646	0.524
17	92.3	2.85	2.38	0.654	0.530
18	84.6	3.00	2.49	0.669	0.545
19	84.6	3.08	2.56	0.669	0.541
20	84.6	3.00	2.63	0.739	0.547
21	84.6	3.00	2.52	0.708	0.541
22	84.6	3.15	2.68	0.677	0.556
23	84.6	2.54	2.13	0.677	0.487
24	84.6	2.69	2.34	0.654	0.520
25	84.6	2.85	2.43	0.685	0.517
26	84.6	3.00	2.42	0.662	0.513
27	84.6	2.92	2.53	0.700	0.542
28	92.3	2.85	2.27	0.631	0.521
29	92.3	3.00	2.42	0.654	0.507
Mean	81.2	2.94	2.39	0.640	0.521
Species ¹	100	3.46	2.88	0.640	0.557

¹The species level statistics were based on all individuals.

Table 4: Distribution of total gene diversity (H_T) within and among the 29 accessions of Russian wildrye using Nei's gene diversity statistics

Locus	H_T	H_S	D_{ST}	G_{ST}	F_{IS}	F_{IT}	F_{ST}	N_m
<i>Aat-1</i>	0.505	0.363	0.142	0.282	-0.008	0.274	0.279	0.645
<i>Aat-2</i>	0.491	0.443	0.048	0.099	-0.067	0.038	0.099	2.288
<i>G6pd-1</i>	0.730	0.650	0.080	0.110	-0.354	-0.205	0.110	2.027
<i>G6pd-2</i>	0.494	0.485	0.008	0.017	-0.812	-0.782	0.017	14.832
<i>Mdh</i>	0.003	0.003	0.000	0.048	-0.053	-0.002	0.048	4.920
<i>Me</i>	0.782	0.681	0.101	0.129	-0.469	-0.279	0.129	1.684
<i>Pgd-1</i>	0.757	0.692	0.065	0.085	-0.212	-0.111	0.084	2.741
<i>Pgd-2</i>	0.730	0.634	0.096	0.131	-0.266	-0.011	0.126	1.732
<i>Skd</i>	0.756	0.672	0.085	0.112	-0.142	0.015	0.112	1.990
<i>Est-1</i>	0.522	0.490	0.032	0.060	-0.380	-0.302	0.056	4.183
<i>Est-2</i>	0.773	0.720	0.053	0.068	-0.359	-0.266	0.068	3.408
<i>Pod-1</i>	0.050	0.044	0.006	0.121	0.300	0.384	0.121	1.823
<i>Pod-2</i>	0.680	0.553	0.126	0.186	-0.122	0.028	0.133	1.627
Mean	0.559	0.495	0.065	0.111	-0.293	-0.152	0.110	2.033

diversity and F -statistic for the 29 accessions are shown in Table 4. Locus *Me* had the highest H_T value, 0.782, whereas locus *Mdh* had the lowest value, 0.003. We also used Wright's F -statistics to characterize the diversity structure. The F_{IT} value was negative (-0.152) which means that there were on average more heterozygosity in the populations than expected (H-W) in the total population based on average allele frequencies across populations. The F_{ST} value was 0.110 and this value was similar to the value of G_{ST} , which ranged from 0.017 for locus *G6pd-2* to 0.282 for locus *Aat-1*. The average value of G_{ST} was 0.111 and this value means that 88.9% was found within the accessions and 11.1% occurred among the accessions. Because F_{ST} and G_{ST} were used to estimate the diversity structure in the present study, these two values indicate that diversity within the accessions was approximately ninefold greater than the genetic diversity among populations.

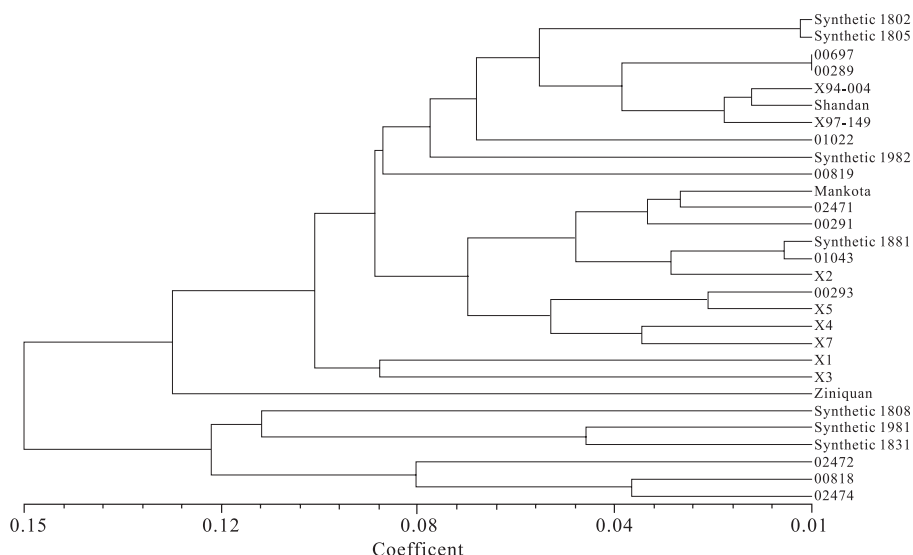
Genetic distance and UPGMA dendrogram

The UPGMA cluster analysis of genetic distance is shown in Fig. 1. Large variation in genetic identity ($I = 0.749-0.990$) and genetic distance ($D = 0.010-0.289$) was observed (data not shown). The 29 accessions were separated into two distinct groups. Group 1 consisted of 23 accessions and was separated into two main branches. Most of the accessions that originated from China fell into group 1. Accession 28, cultivar Ziniquan domesticated in Xinjiang, China, stood alone in group 1. Accession 1 (synthetic 1802), accession 2 (synthetic 1805), accession 12 (00697) and accession 14 (00289) were clustered together and had high genetic identity values. Group 2 consisted of six accessions of which three synthetic accessions (synthetic 1808, synthetic 1981, synthetic 1831) and accession 13 (02474) originated from Canada.

Discussion

In our study, the accessions of Russian wildrye maintained a high level of genetic diversity at both the species and population levels. The number of polymorphisms at the species level (P_s), expressed as a percentage, was 100% and at the population level (P_p) was 81.2%. These values for extent of polymorphisms were much higher than those reported by Hamrick and Godt (1989) for other outcrossed and wind-pollinated plant species ($P_s = 66.1\%$, $P_p = 49.7\%$) and other temperate-zone species ($P_s = 48.5\%$, $P_p = 32.6\%$). The

Fig. 1: Cluster dendrogram based on Nei's (1978) unbiased genetic distance for 29 accessions of Russian wildrye using the unweighted pair group method with arithmetic mean



mean expected heterozygosity (H_e) and mean number of alleles per locus (A) were 0.521 and 2.944, respectively, for the 29 Russian wildrye accessions. These values were higher than the values reported by Hamrick and Godt (1989) for widespread species ($H_e = 0.159$, $A = 1.72$). We propose two reasons could account for this high level of genetic diversity for Russian wildrye. Firstly, it may be related to its ability to cross-pollinate: the highly self-sterile character of outcrossed species leads to greater genetic diversity than that in self-pollinated species (Brown 1979, Hamrick and Godt 1989, 1996). Secondly, it may be related to its longevity character. It has been reported that long-lived perennial species maintain higher levels of genetic variability than annuals and short-lived perennials (Hamrick et al. 1992).

Genetic diversity within accessions was much higher than the genetic diversity among populations. The proportion of the total genetic diversity partitioned among the 29 accessions (G_{ST}) was 0.1114, a value which is similar to the G_{ST} of 0.099 for other wind-pollinated, outcrossed species reported by Hamrick and Godt (1989). In their review of plant allozymes, Hamrick et al. (1992) and Chung and Hamrick (1991) reported that the extent of genetic diversity found within and among populations is dependent on the geographical distribution of the species, the method of seed dispersal and the mode of pollination. A greater extent of the total genetic diversity for outcrossed species, such as Russian wildrye, is found within populations than among populations (Hamrick and Godt 1996). A relatively high gene flow value ($N_m = 2.0333$) also suggests that a low level of genetic diversity is present among the populations. Gene flow can be affected by selection, genetic drift, mutation and the dispersed amount of pollen. The high level of outcrossing, which occurs in Russian wildrye (Jensen et al. 1990) would enhance gene flow, and thereby promote genetic diversity within populations. A high level of genetic diversity within populations of outcrossed species has been reported by Wei et al. (1996), Lopez-Pujol et al. (2001) and Batista and Pedro (2002).

The frequency of the 46 alleles in the 29 Russian wildrye accessions varied extensively. Some alleles were ubiquitous, whereas some alleles were common or rare. This pattern of allele frequency and distribution in the 29 accessions is similar to the description of allozyme frequency and distribution that

was reported in wheat (Marshall and Brown 1981). The genetic diversity of four of the eight enzymes analysed in the present study (G6PD, MDH, SKD and PGD) was estimated by Wei et al. (1996). The enzymes G6PD and MDH, were detected at one locus and two loci, respectively, in the Wei study, whereas, these two enzymes were detected at two loci and one locus, respectively, in present study.

We found three rare alleles, *aat-1c*, *mdhb* and *est-1d*, in accessions 19, 26 and 29. These three accessions were wild accessions collected from Xinjiang, China. These three alleles have not been reported previously. Raelson and Grant (1989) and Machado et al. (1993) reported that differences in the environment under which plants are grown can affect the detection of allozyme alleles for the same enzymes. The accessions used in the two studies have different origins and genetic backgrounds and therefore, one would expect variation in the allozyme alleles.

The three rare alleles were probably not detected in the synthetic accessions and cultivars because of domestication and selection resulting in the loss of some unusual traits (Li et al. 2005). The emergence of these rare alleles may be related to the environments from which the accessions were collected and their distinct morphologies (Fadhel and Boussaïd 2004). Therefore, we propose that these rare alleles should be further analysed according to their morphological or agronomical traits. Those accessions, which possess these rare alleles, should be prioritized for conservation and phenotypic evaluation and their areas of origin should be targeted for further germplasm collection.

The cluster dendrogram separated the 29 accessions into two groups according to the genetic distance among the accessions. The Ziniquan cultivar, accession 28, was genetically most distant from the other accessions. This finding suggests that selection may have resulted in the creation of unique genotypes which are genetically distant from the other accessions. The accessions, which originated from China, were in the same group and this clustering indicated that a high level of genetic identity within this group exists (Wei et al. 1996, Huh 2001, Fu and Dane 2003).

Allozyme analyses revealed a large number of polymorphic loci among 29 accessions of Russian wildrye. High levels of genetic diversity were evident within accessions. Rare alleles,

which have not been reported in previous studies, were detected in several accessions. The accessions, which carry these rare alleles, trace their origin to sites in Xinjiang, China, where Russian wildrye is indigenous. The accessions with rare alleles require additional evaluation to determine whether they possess unique phenotypic traits which would be useful in applied plant breeding. Further germplasm collections should be conducted at the sites at which the accessions with rare alleles originated. Additional germplasm collection at diverse sites would be valuable to provide plant breeders with accessions which differ widely in their genetic background.

Acknowledgements

We are indebted to Prof. Jinzhong Zhu and Gaofeng Wang for their help in locating and collecting materials in Xinjiang, China; Zhiyong Li for providing the accessions; Zhan Shan for help in the laboratory experiments and Dr Cheng Fang for his suggestions.

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